22 November 2011
EMA/HMPC/430506/2009
Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Olea europaea* L., folium

Based on Article 16d(1), Article 16f and Article 16h of Directive 2001/83/EC as amended (traditional use)

Final

Herbal substance(s) (binomial scientific name of the plant, including plant part)	***Olea europaea* L. folium (olive leaf)**
Herbal preparation(s)	Herbal substance Fresh or dried leaves Herbal preparations Comminuted herbal substance (dried leaves) Powdered herbal substance (dried leaves)
Pharmaceutical forms	Herbal substance and comminuted herbal substance as herbal tea for oral use. Herbal preparation in solid dosage form for oral use.
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1. Introduction

The aim of this report is to assess available preclinical, clinical and other relevant data on Oleae folium for preparing a Community monograph. This report is based on the documentation provided by the European Medicines Agency (EMA) completed by additional searches and information taken from recent international literature on *Olea europaea* L., Oleae folium.

1.1. Description of the herbal substance(s), herbal preparation(s) or combinations thereof

Olea europaea L. belongs to the Oleaceae family. The name *Olea europaea* L. synonym with *O. officinarum* CRANTZ; *O. pallida* SALISB applies to both the wild *O. europaea* ssp. *sylvestris* (MILLER) ROUY (syn *O. oleaster* HOFFM. et LINK, *O. sylvestris* MILL.) and domestic (cultivated) plant which is mainly known as *O. europaea* ssp. *sativa* (HOFFM et LINK) ROUY (syn = *O. europaea* L. var. *europaea*, *O. europaea* ssp. *sativa* LOUD., *O. europaea* L. ssp. *sativa* ARCANG., *O. gallica* MILL., *O. hispanica* MILL., *O. lancifolia* MOENCH, *O. sativa* GATERAU). Several varieties have been recognised. More than 300 are differentiated, among which more than 150 only in Italy for oil or table-olives production (Blaschek *et al.* 2006).

The olive tree is an evergreen that grows to approximately 6-9 metre in height. It is native to the southern European countries and throughout the entire Mediterranean region as far as Iran and beyond the Caucasus. Olive trees are also cultivated in similar climate zones in the Americas. Leaves are 7.5 cm long, narrow opposite, lanceolate or linear, with entire margins and acute tips, silver-green (grey green) on top, the underside lighter, containing fine white, scale-like hairs. The leaves are gathered throughout the year.

Latin Name: *Olea europaea* folium (Oleaceae); olive leaf (English), Feuilles d'Olivier (French), Ölbaumblätter, Olivenblätter (German), Foglie di olivo (Italian), Hojas de olivo (Spanish), folhas de oliveira (Portuguese); Olijfblad (Dutch), liść oliwki (Polish), Φύλλα Ελιάς (Greek)

- Herbal substance(s)

O. europaea L., folium is the dried leaf of the plant containing minimum 5% of oleuropein ($C_{25}H_{32}O_{13}$; Mr 540.5) (Ph. Eur. 2008:1878).

The leaf is simple, thick and coriaceous, lanceolate to obovate, 30-50 mm long and 10-15 mm wide, with a mucronate apex. The upper surface is greyish-green, smooth and shiny, the lower surface paler and pubescent (Ph. Eur. 2008:1878), Ph. Belg. V, Ph. Fra. IX, Ned F. 6, (Van Hellemont 1986).

The leaves are harvested from cultivated trees and dried in the shade. The crude herbal drug complies with the European Pharmacopoeia monograph "Oleae folium" 01/2009:1878. The drug tastes bitter. It can be identified by its microscopic characteristics, particularly the presence of many shield-shaped covering trichomes and sclerites clearly visible in the powder; these are long, have thick walls, are bent here and there, are highly refringent and end as if they were truncated. These characteristics allow verification of the identity of the drug, which in addition, is characterised by the presence of triterpenes (by the red colour developed by an ether extract in the presence of acetic anhydride and sulphuric acid). The assay includes thin layer chromatography to show the presence of oleuropein (Bruneton 1999).

Constituents of olive leaves

- Iridoid monoterpenes: including among others, oleuropein (5-9%), additionally 6-O-oleuropeine saccharose, ligstroside, oleoroside etc.
- Triterpenes: including oleanolic acid, maslinic acid etc.
- Flavonoids: luteolin, kaempferol, chrysoeriol and apigenin derivatives etc.
- Chalcones: olivine, olivine-4'-O-diglucoside etc. (PDR for Herbal Medicines 2007).
- Phenolic acids: cumaric acid, caffeic acid, ferulic acid, vanillic acid etc.
- Coumarins: aesculetin, scopoletin, aesculins.

Additional analytical information:

The main constituents of olive leaves are secoiridoids like oleuropein, ligstroside, I methyloleuropein, and oleoside (Gariboldi *et al*. 1986) as well as flavonoids (apigenin, kaempferol, luteolin, chrysoeriol) and phenolic compounds (caffeic acid, tyrosol, hydroxytytrosol) (Ross 2005).

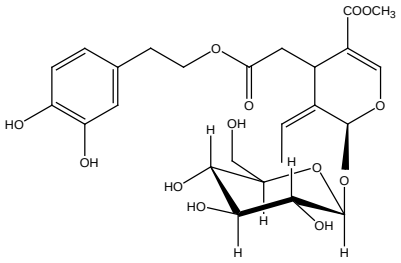
Two new phenolic compounds were isolated from fruits of *O. europaea*, Hojiblanca cultivar. The first compound is the methyl acetal of the aglycone of ligstroside, while the second derivative, not yet reported in the literature, is the β-hydroxytyrosyl ester of methyl malate. These microcomponents may be responsible for hedonistic-sensorial characteristics of olive products (Bianco *et al*. 2006).

The secoiridoids is a very specific group that are abundant in Oleaceas and many other plants that are produced from the secondary metabolism of terpenes as precursors of various indole alkaloids and are usually derived from the oleoside type of glucoside oleosides, which are characterised by a combination of elenolic acid and a glucosidic residue. It can be stated that these compounds proceed from the acetate/mevalonate pathway (Gariboldi *et al*. 1986).

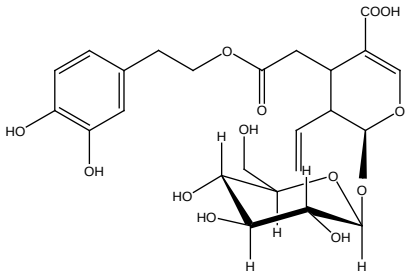
Oleuropein **1**, the major constituent of the secoiridoid family in the olive trees, is a complex phenol present in large quantities in olive tree leaves, in low quantities in olive oil (Soler-Rivas *et al*. 2000) and is responsible for the bitter taste and pungent aroma of olive oil. It has been discovered in 1908 by Bourquelot and Vintilesco, and its structure was specified as being that of a heterosidic ester of elenolic acid and dihydroxyphenylethanol by Panizzi *et al.* 1960, with the empirical formula $C_{25}H_{32}O_{13}$ (Pannizi *et al*. 1960). Oleuropein can be hydrolyzed to hydroxytyrosol, elenolic acid, oleuropein aglycone and glucose (Manna *et al*. 2004). Two of its by-products are also present in the olive plant together with oleuropein **1** and the mono-demethyl-derivatives **2** and **3**. Compound **2** is demethyl-oleuropein, which differs from oleuropein **1** in having a free carboxylic group on the pyranosic ring. Compound **3** is the oleoside methyl ester, known also as a glucoside of the elenolic acid, in which the carboxyl that esterifies the dihydroxy-phenyl-ethanol in the oleuropein **1** is here the free functionality. The two acid compounds **2** and **3** are two indicators of maturation of the olive. Their relative quantity, as regards to the oleuropein **1**, increases in fact as soon as the maturation proceeds, while the quantity of oleuropein decreases. This datum is in connection with the increase of the activity of the hydrolytic enzymes with the progress of the maturation, particularly to the activity of the esterases, responsible of the hydrolysis of the two ester bonds of the oleuropein (Amiot *et al*. 1989). The ligstroside **4** (Asaka *et al*. 1972) differs from the oleuropein **1** in the presence of a tyrosol residue instead of dihydroxy-phenyl-ethylic alcohol. The dimethylester of the oleoside **5**, also as glucoside of the methylester of the elenolic acid, contains the two acidic functions of the oleuropein esterified with the residue of methanol (Gariboldi *et al*. 1986). The oleuroside **6** is an isomer of the oleuropein, differing from **1** in the exocyclic double bond position and its structure was determined as secoxyloganin 3,4-dihydroxyphenethyl ester (Kuwajima *et al*. 1985; Khan *et al*. 2007).

Triterpenes have been also isolated like maslinic acid, β-amyrin, oleanolic and maslinic acid, the occurrence of maslinic acid in fresh leaves of *O. europaea* strongly supports it is a true metabolite of the plant. Recently it has been reported that maslinic acid is produced, during the ageing of olive husks, possibly through microbial α-hydroxylation of oleanolic acid. Furthermore, to their knowledge, this appears to be the first record of isolation of β-amyrin in *O. europaea* (Mussini *et al*. 1975). Also, several alkaloids have been determined in the leaves of *Olea* like cinchonine and cinchonidine derivatives (Bezanguer-Beauquesne *et al*. 1990; Ross 2005).

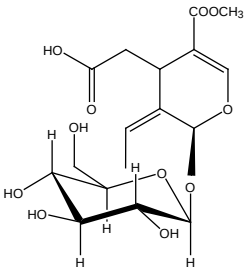
Olive leaves contain around 60-90 mg/g (dry weight) oleuropein, (Le Tutour *et al*. 1992) plus significant levels of a glucosidic ester of elenolic acid and hydroxytyrosol (3,4-dihydrophenylethanol). However, it turns out that oleuropein and the products of its hydrolysis, oleuropein aglycone, elenolic acid, beta-3,4-dihydroxyphenyethyl alcohol and methyl-o-methyl elenolate, are the major molecules of interest biologically (Fleming *et al*. 1973).



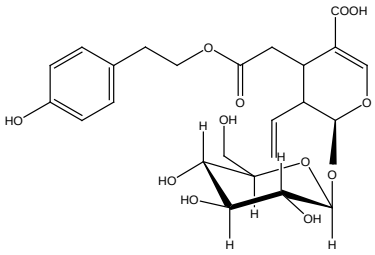
1 oleuropein



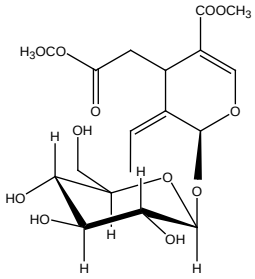
2 demethyl-oleuropein



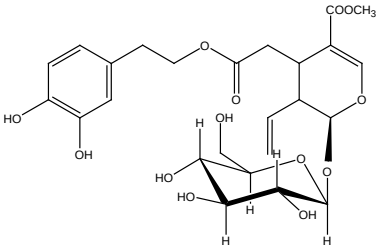
3 oleoside methyl ester



4 ligstroside



5 dimethylester of the oleoside



6 oleuroside

- Herbal preparation(s)

Olive leaf extract is derived from the leaves of the olive tree. The olive leaf dry extract complies with the European Pharmacopoeia monograph “Oleae folii extractum siccum” 04/2009:2313 of European Pharmacopoeia.

Olive leaf and extracts are utilised in the complementary and alternative medicine community for its perceived ability to act as a natural pathogens killer by inhibiting the replication process of many pathogens. Olive leaf is commonly used to fight colds and flu, yeast infections, and viral infections such as the hard-to-treat Epstein-Barr disease, shingles and herpes. Olive leaf is also good for the heart, has shown to reduce low-density lipoproteins (LDL) while through different studies have been published that olive leaf lowers blood pressure and increases blood flow by relaxing the arteries (Ross 2005).

- Combinations of herbal substance(s) and/or herbal preparation(s) including a description of vitamin(s) and/or mineral(s) as ingredients of traditional combination herbal medicinal products assessed, where applicable.

Oleae folium is reported to be used in combinations with *Rauwolfia* (Holzhauer & Knobloch 1950), *Veratrum* or with *Viscum album*.

This assessment and the monograph refers exclusively to the use of Oleae folium as a single ingredient.

Vitamin(s)[1]: not applicable

Mineral(s)[1]: not applicable

1.2. Information about products on the market in the Member States

The following information has been received on products in European Union:

France

Traditional use

Preparations: Powdered dried leaves since 1980

Pharmaceutical form: Hard capsules

Posology for oral use in adults: 3-5 times daily (containing 275 mg powder each)

Indications: Traditionally used to promote urinary and digestive elimination functions.

Traditionally used to promote the renal elimination of water.

Germany

Traditional use

Preparations: extract (1:0.71-0.86), extraction solvent: ethanol 96% V/V

dry extract (7.9-12:1), extraction solvent: ethanol 96% V/V at least since 1976

Pharmaceutical form: oral liquid and coated tablet

Posology for oral use in adults:

30-50 drops, 3 times daily
(or 2 times daily 45-75 drops) 100 g (= 98 ml) liquid contain 18.2 g extract, 1 g = 28 drops

Daily dose: 3-5 coated tablets containing 14 mg dry extract each divided into 2-3 single doses

Indications: Traditionally used to support the cardiovascular system.

[1] Only applicable to traditional use

Spain

Preparations:

Powdered or cut leaves for oral use as herbal tea or

Powdered or cut leaves for oral use in capsules (210-400 mg) up to 3 times daily since 1986

Posology for oral use in adults: 3 times daily

Indications: Traditionally used to enhance the renal excretion of water and to support the cardiovascular system.

Regulatory status overview

Member State	Regulatory Status				Comments
Austria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No product registered
Belgium	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Bulgaria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Cyprus	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Czech Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No product registered
Denmark	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No product registered
Estonia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Finland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
France	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	Powdered dried leaves since 1980
Germany	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	extract (1:0.71-0.86), extraction solvent: ethanol 96% V/V dry extract (7.9-12:1), extraction solvent: ethanol 96% V/V since 1976
Greece	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No product registered
Hungary	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Iceland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Ireland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Italy	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	No registered or authorised THMP/HMP
Latvia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Liechtenstein	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Lithuania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Luxemburg	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Malta	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known

Member State	Regulatory Status				Comments
The Netherlands	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Norway	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Poland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Portugal	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No product registered
Romania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Slovak Republic	☒ MA	☐ TRAD	☒ Other TRAD	☐ Other Specify:	Not known
Slovenia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Spain	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	Powdered dried leaves since 1986
Sweden	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No product registered
United Kingdom	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known

MA: Marketing Authorisation
TRAD: Traditional Use Registration
Other TRAD: Other national Traditional systems of registration
Other: If known, it should be specified or otherwise add 'Not Known'
This regulatory overview is not legally binding and does not necessarily reflect the legal status of the products in the MSs concerned.

1.3. Search and assessment methodology

Search terms:

Databases: Pubmed, Medline, HealLink, Scopus were searched with the search term *Olea europea* L., olive leaf, hydroxyl-tyrosol, oleuropein

Libraries: University of Athens, Laboratory of Pharmacognosy and Chemistry of Natural Products of the University of Athens

2. Historical data on medicinal use

2.1. Information on period of medicinal use in the Community

The leaves of the olive tree *O. europaea* have been widely used in folk medicine in regions around Mediterranean Sea and the islands therein (Bouaziz and Sayadi, 2005).

Olive leaf and extracts are utilised in the complementary and alternative medicine community for its ability to act as a natural pathogens killer by inhibiting the replication process of many pathogens (Juven and Henys 1972). Olive leaf extract has been also used as a folk remedy for combating fevers and other diseases, such as malaria, while several reports have shown that it has the capacity to lower blood pressure in animals, to increase blood flow in the coronary arteries, to relieve arrhythmia and to prevent intestinal muscle spasms (Samuelson 1951; Zarzuelo 1991).

Interest in the potential benefits of extracts from the olive tree originates from two main independent historical sources. The first formal medical mention of the olive leaf, an account describing its ability to cure severe cases of fever and malaria, occurred about 150 years ago. In 1854, the Pharmaceutical Journal published a report by Daniel Hanbury. The author wrote he discovered the effective tincture in 1843 and had used it successfully. As second source appear records that, in the early 19th century,

Spanish physicians sometimes prescribed olive leaves as a “febrifuge”, and consequently, during the Spanish war of 1808-1813, the French Officers de Santé often used them to treat cases of “intermittent fever”. This method became well known in England for treating sick Britons returning from tropical colonies. The author believed that a bitter substance in the leaves was the key healing ingredient (Cruess & Alsberg 1934; Samuelsson *et al*. 1951; Veer *et al*. 1957).

Decades later, scientists isolated a bitter substance from the leaf and named it oleuropein. It was reported that it makes the olive tree particularly robust and resistant against insect and bacterial damage. Oleuropein is an iridoid, a structural class of chemical compounds found in plants. It is present in olive oil, throughout the olive tree, and is the bitter material that is eliminated from the olives when they are cured. In 1962, an Italian researcher reported that oleuropein lowered blood pressure in animals (Panizzi L. *et al*. 1960). This triggered of scientific interest in the olive leaf. Other European researchers confirmed this interesting finding. In addition, they found it could also increase blood flow in the coronary arteries, relieve arrhythmias, and prevent intestinal muscle spasms (Petkov & Manolov 1972; Juven & Henys 1972; Kubo & Matsumoto 1984). The dried or fresh leaves have been also used against malaria as antipyretic as well as diuretic.

France: “Feuille d’olivier” Olive leaf is regarded as one of the herbal drugs whose efficacy and safety has been proven by thorough literature studies and long-term traditional use (Agence du Medicament, Paris 1998) stating the use of olive leaf as digestive and as mild diuretic (pour faciliter les functions d’élimination urinaire et digestive., pour favoriser l’élimination renale d’eau) (Oliviase UPSA Fr for diuresis) (Martindale 1996). It has also been used in combination with a water extract of Birch.

Germany: Olive leaves have been traditionally used at least since 1976, for prevention of atherosclerosis and against hypertension (Martindale 1996). It has also been used in combination either with Rauwolfia (Holzhauer & Knobloch 1950), Veratrum or with Viscum album.

The Commission E issued a negative monograph (Blaschek *et al.* 2006; Blumental *et al.*1998).

Worldwide, the following information has been received (references available to the Rapporteur):

Arabic countries: In Unani medicine, dried plant is taken by fumigation as an abortifacient.

Argentina: Decoctions of the dried fruit and of the dried leaf are taken orally for diarrhoea and to treat respiratory and urinary tract infections.

Brazil: Herbal tea of the fresh leaves is taken orally to treat hypertension and to induce diuresis.

Bulgaria: Herbal tea of the fresh or dried leaves is taken orally to treat hypertension (Petkov 1979).

Canary Islands: An infusion prepared from the fresh or dried leaf is taken orally as hypoglycaemic agent. Leaves are taken orally as hypotensive and administered per rectum for haemorrhoids.

Cuba: Herbal tea of the fresh leaves is taken orally to treat hypertension

Greece: Hot water extract of the leaf is taken orally for high blood pressure.

Italy: Extract of the fruits essential oil is taken orally as a cholagogue and laxative and to treat renal lithiasis. It is used externally to treat sores, burns, and rheumatism. Water extract of the fruits essential oil is used externally to treat sunburns. Decoction of the fruit essential oil is taken orally as a diuretic and hypotensive. Fruit fixed oil is taken orally as a febrifuge. Infusion of the dried leaf is taken orally as a hypotensive and is used for its anti-hypotensive properties (Mainoli 1951). Infusion of the fresh leaf is taken orally as a hypotensive and applied externally as a vulnerary, emollient for ingrown nails, and restorer of epithelium.

Japan: Hot water extract of the dried bark is taken orally as an antipyretic, for rheumatism, as a tonic and for scrofula.

Kenya: Stem, fresh and dried twigs of *O. europaea* ssp. *africana* are used as a chewing stick.

Madeira: Infusion of leaves of *O. europaea* var. *maderensis* is taken orally as an antihypertensive.

Mexico: Decoction of dried leaves is taken orally for diabetes.

Morocco: Leaves are taken orally for stomach and intestinal disease and used as a mouth cleanser. Essential oil made from the leaves is taken orally for constipation, liver pain and tonic and applied externally for hair care.

Oman: Barks and leaves are applied externally for skin rash. The Cataplasm prepared from leaves is applied externally for ulcers.

Peru: Hot water extract of the dried bark is taken orally for urinary retention, herpes simplex, and constipation and to expel biliary calculi.

Reunion Island: Hot water extract of the dried *O. europaea* ssp. *africana* plant is taken orally for diabetes, diarrhoea, rheumatism, fever and gastroenteritis in infants.

Serbia: (former Yugoslavia) Hot water extract of the dried leaf orally for diabetes. (Ross 2005).

Spain: Infusion of the leaf is taken orally for hypertension. Extracts of the leaf is taken orally for gastrointestinal colic. Leaves are eaten for diabetes.

Tunisia: Extract of the dried leaf is taken orally for diabetes and as hypotensive.

Turkey: The fruit is used externally as a skin cleanser.

Ukraine: Hot water extract of dried plant is taken orally for bronchial asthma.

2.2. Information on traditional/current indications and specified substances/preparations

The following herbal substances and herbal preparations are on the European market for a period of at least 30 years as requested by Directive 2004/24 EC and were proposed for the monograph on traditional use.

Herbal substance

- fresh or dried leaves

Herbal preparations

- comminuted leaves for herbal tea 5-10 g/200 ml, up to three times day (daily dose of 30 g) (Duke 2002; Raynaud 2005).
- powdered dried leaves, 200-400 mg, three times daily.

Liquid extract (1:0.71-0.86) extraction solvent: ethanol 96% V/V.

Dry extract (7.9-12:1), extraction solvent: ethanol 96% V/V.

Indications of the traditional herbal substance and preparations of Olive leaves.

Indication:

Traditional herbal medicinal product according the European market overview:

- to support cardiovascular function (Germany);
- to enhance the renal excretion of urine (France);

- herbal medicinal product for the relief of functional cardiovascular complaints (Spain);
- cardiovascular system (in France and Spain).

After discussions in both MLWP and HMPC it has been acknowledged that the tradition and the pharmacologically plausible threefold mild activity (diuretic, antidiabetic, anti-hypercholesterolaemic) would be considered as beneficial for the cardiovascular system (function). Safety concerns were raised towards the demarcation between mild functional complaints and organic symptoms. Patients may be encouraged for self treatment, where clearly medical supervision and medically supervised medication is required. Therefore, the HMPC endorsed by majority the diuretic indication only:

Traditional herbal medicinal product used to promote the renal elimination of water, in mild cases of water retention.

Herbal preparations in solid dosage forms for oral use or comminuted herbal substance as herbal tea for oral use.

After the acceptance of the above referred indication, the HMPC agreed that products used for more than 35 years in Germany (liquid extract (1:0.71-0.86) ethanol 96% V/V) and dry extract (7.9-12:1) ethanol 96% V/V.) should be excluded from the monograph as the indication which is in use in Germany "…to support the cardiovascular system" does not comply with the one above.

2.3. Specified strength / posology / route of administration / duration of use for relevant preparations and indications

Posology:

Adults and elderly

- fresh or dried leaves

Up to 20 g of fresh or up to 10 g of dried olive leaves in 300 ml of water, boiled, till the water to reach 200 ml, filter. To be consumed hot twice a day (morning and evening) (Van Hellemont 1986).

- comminuted or powdered dried leaves for herbal tea (Duke 2002; Raynaud 2005).

7-8 g dry leaf in 150 ml water, 3-4 times daily or 2 teaspoons leaf in hot water, steep for 30 minutes (Duke 2002).

6-10 g of dried plant, 1-3 doses per day (extract corresponding to 600 mg dry aqueous extract) (Van Hellemont 1986).

Powdered dried leaves, 200-275 mg, 3-5 times daily (maximum daily dose of 600-1375 mg).

Duration of use

Up to 2-4 weeks.

3. Non-Clinical Data

3.1. Overview of available pharmacological data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

PRIMARY PHARMACODYNAMICS

O. europaea, and its products and chemical constituents have been recognised as important components of a healthy diet because of their phenolic content (Visioli *et al*. 2002). The olive leaf

extract is used to enhance the immune system, as an antimicrobial, antiviral, as an antioxidant, hypoglycaemic agent and for use in cardiovascular problems (PDR for Herbal Medicines 2007).

***In vitro* studies**

Antimicrobial activity

Dried leaf extracts (ethanol:water 1:1) at concentrations of 500 mg/ml, were found to be inactive *in vitro* against *Aspergillus fumigatus, A. niger, Fusarium oxysporum, Penicillium digitatum, Rhizopus nigricans, Trichophyton mentagrophytes, Candida albicans* and *Saccharomyces pastorianus* (Guerin & Reveillere 1985).

Activity against *Mycobacterium tuberculosis* (H37Rv TMC 102) of 95% ethanol extracts of *O. europaea* (part not specified) has been reported, using the broth culture method (Grange & Davey 1990).

Hot water extracts of olive leaf of Argentinian origin, at a concentration of 62.5 mg/ml, were found to be inactive against *Staphylococcus aureus, Aspergillus niger* and *Escherichia coli* (agar plate method) (Anesini & Perez 1993).

Hot water leaf extracts (1 mg/ml) were inactive against *Salmonella typhi* (Perez & Anesini 1994).

Olive leaves are known to resist insect and microbial attack, and *in vitro* studies have been conducted to establish the range of activity of olive leaf extracts. Olive leaf extract has been reported to be an effective antimicrobial agent against a variety of pathogens, including *Salmonella typhi, Vibrio paraemoliticus* and *Streptococcus aureus* (including penicillin-resistant strains) and *Klebsiella pneumoniae* as well as *E. coli* (Caturla *et al*. 2005; Bisignano *et al*. 1999).

An aqueous extract of olive leaf was bactericidal against *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli* and *Staphylococcus aureus* (0.6% w/v), as well as bacteriostatic against *Bacillus subtilis* (at 20% w/v) (Markin & Duek 2003).

Antiviral activity

In vitro antiviral activity of an olive leaf extract (not further defined) against HIV-1 virus (infected H9 T lymphocytes) has been demonstrated in cell culture (IC_{50} 0.2 mcg/ml). Cell-to-cell transmission of HIV was inhibited in a dose-dependent manner and HIV-1 replication was inhibited in an *in vitro* experiment (Lee-Huang *et al*. 2003). It was shown that the olive leaf has an interesting effect on *Herpes simplex* virus-1 (HSV-1) *in vitro*. The *in vitro* virucidal effect of olive leaf extract on HSV-1 in concentrations >1 mg/ml has been proven. The CC_{50} (50% cytotoxic concentration) of olive leaf extract for Vero cells and IC_{50} were 1.75 and 0.65 mg/ml, respectively. When applied to cell culture infected with HSV-1, one hour before challenge, olive leaf extract showed no antiviral activities. When applied to the cells followed by the virus infection 1 hour later, or to the media containing the virus and the combination was added to cell culture 1 hour later, olive leaf extract showed anti HSV-1 activities at concentrations >1 mg/ml (Motamedifar *et al*. 2007).

The virucidal activity of olive leaves is more likely to be attributed to its ability to prevent virus entry into the cells. It may be due to the interaction of olive leaf extract with Vero cell membrane and/or HSV-1 envelope. The exact mechanism of olive leaf extract's antiviral activity is still not clear. However, it might be attributed to the prevention of attachment and absorption of virus particles to the cell and thereby blockade of their entry to the cells. In agreement with this hypothesis, olive leaves extract was shown to interact with the surface of phospholipid bilayer (Khan *et al*. 2007). Moreover it has been shown that OLHE is a viral inhibitor at early stages of replication, probably via blocking of viral envelope fusion (Micol *et al*. 2005).

Antiplatelet activity

Olive leaf has been reported to inhibit platelet aggregation and production of thromboxane A2 (a stimulator of platelet aggregation with vasodilatory effects) (Petroni *et al*. 1995).

The effects on PRP aggregation of oleuropein, another typical olive oil phenol, and of selected flavonoids (luteolin, apigenin, quercetin) were also tested and found to be much less active. On the other hand a partially characterised phenol-enriched extract obtained from aqueous part from olive oil showed rather potent activities. These results were the first evidence that components of the phenolic fraction of olive oil can inhibit platelet function and eicosanoid formation *in vitro*, and that other, partially characterised, olive derivatives share these biological activities.

Also of interest is a recent study reporting that olive leaf extract inhibited both angiotensin converting enzymes (Hansen *et al*. 1996).

Antioxidant activity

Experiments have been conducted to demonstrate the antioxidant activity of olive leaf extracts. In rat epithelial cells stimulated with cytokines, an olive leaf polyphenol concentrate extract reduced nitrite concentration and free radical production. The effects of several natural antioxidants on nitric oxide modulation and oxidative status were determined in rat epithelial lung cells. Resveratrol and olive leaf polyphenol concentrate extract were found to be effective in reducing nitrite levels, modifying nitric oxide mRNA, and decreasing free radical production. In particular resveratrol and olive leaf polyphenol concentrate extract, may have therapeutic potential in the treatment of inflammatory diseases (Zaslaver *et al*. 2005).

Recently (Fleming *et al.* 2011) an *in vitro* study, kinetic measurements was performed with an 80% ethanolic extract of olive leaf and its possible inhibitory effects on xanthine oxidase, an enzyme well known for its significant contribution to the pathological process of gout. The studied standardised extract significantly inhibited the activity of xanthine oxidase. Through this study the authors suggest to provide a rational basis for the use of olive leaves against gout in Mediterranean folk medicine.

Effects on the inflammatory response

Fresh olive leaf extracts of Italian provenance were assessed *in vitro* for effects on the complementary system both alternative and classical pathways. Neither ethyl acetate (50 mcg/ml) nor methanol (50 mcg/ml) extracts inhibited the alternative pathway while both inhibited the classical pathway, at IC_{50} >7.7 µ/ml (EtOAc) and >5.8 µ/ml (MeOH) (Pieroni *et al*. 1996).

Hypoglycaemic activity

The inhibitory action of the olive leaf ethanol extract on the activities of human amylases was examined *in vitro*. Olive leaf ethanol extract inhibited the activities of α-amylase from human saliva and pancreas with IC_{50} values of 4 and 0.02 mg/ml, respectively (Komaki *et al*. 2003). This finding is due to the inhibitory action of the flavonoids (luteolin-7-O-b- glucoside and luteolin-4'-o-b-glucoside) as well as of the triterpene oleanolic acid on α-amylase from human saliva and pancreas (Komaki *et al*. 2003)

***In vivo* studies**

Although tradition attributes to the olive tree leaf numerous properties (febrifuge, hypoglycaemic, hypotensive, diuretic, and more) few of them have been studied experimentally.

Antiviral activity

Animal experiments studies suggest olive leaf extracts possess antiviral activity against viral haemorrhagic septicaemia rhabdovirus (VHSV) (Micol *et al*. 2005).

Oleuropein has been claimed in a United States patent to have potent antiviral activities against DNA and RNA viruses such as herpes mononucleosis, hepatitis virus, rotavirus, bovine rhinovirus, canine parvovirus, and feline leukaemia virus (safe and effective natural antiviral agents, the antiviral activity of a commercial extract of olive leaves *O. europaea*, and its major component, oleuropein, were tested against a model rhabdovirus such as the VHSV, which infects continental and sea farmed fish and a wide range of wild marine species in Europe, North America and Japan. The results presented showed the inhibitory action of both extract and oleuropein against VHSV when the virus was incubated with the agents before infecting the cells, suggesting a direct inactivation effect on VHSV infectivity by the compounds.

Hypolipidemic activity

Antihypercholesterolaemic activity has been shown in rats given a high daily dose, administered intragastrically of 500 mg/kg of a glycerine: ethanol leaf extract for 15 days. Activity was shown both in diet-induced and triton-induced hypercholesterolaemic animals (De Pasquale *et al*. 1991).

Oleuropein-rich extracts from olive leaves and their enzymatic and acid hydrolysates respectively rich in oleuropein aglycone and hydroxytyrosol, were prepared under optimal conditions. The antioxidant activities of these extracts were examined by a series of models *in vitro* (superoxide dismutase (SOD) and catalase (CAT) activities were evaluated in liver tissue). In this study the lipid-lowering and the antioxidative activities of oleuropein, oleuropein aglycone and hydroxytyrosol-rich extracts in rats fed a cholesterol-rich diet were tested. Wistar rats fed a standard laboratory diet or cholesterol-rich diets for 16 weeks were used. The serum lipid levels, the thiobarbituric acid reactive substances (TBARS) level, as indicator of lipid peroxidation, and the activities of liver antioxidant enzymes (SOD and CAT) were examined. The cholesterol-rich diet induced hyperlipidaemia resulting in the elevation of total cholesterol (TC), triglycerides (TG) and low-density lipoprotein cholesterol (LDL-C). Administration of polyphenol-rich olive leaf extracts significantly lowered the serum levels of TC, TG and LDL-C and increased the serum level of high-density lipoprotein cholesterol (HDL-C). Furthermore, the content of TBARS in liver, heart, kidneys and aorta decreased significantly after oral administration of polyphenol-rich olive leaf extracts compared with those of rats fed a cholesterol-rich diet. In addition, these extracts increased the serum antioxidant potential and the hepatic CAT and SOD activities. The CAT and SOD activities significantly decreased in livers of rats fed a cholesterol-rich diet compared to those fed a control diet. The decrease was significantly restored ($P<0.05$) in the presence of the olive leaves and the hydrolysate extracts. These results suggested that the hypocholesterolemic effect of oleuropein, oleuropein aglycone and hydroxytyrosol-rich extracts might be due to their abilities to lower serum TC, TG and LDL-C levels as well as slowing the lipid peroxidation process and enhancing antioxidant enzyme activity (Jemai *et al*. 2008).

Besides, the hypothesis *in vitro* by inducing LDL oxidation with copper sulphate and pre-incubating the samples with oleuropein, the bitter principle of olives, that is one of the major components of the polyphenolic fraction of olive oil. Oleuropein 10^{-5} M effectively inhibited $CuSO_4$-induced LDL oxidation, as assessed by various parameters. It has been demonstrated that polyphenolic components of the Mediterranean diet interfere with biochemical events that are implicated in atherogenetic disease, thus proposing a new link between the Mediterranean diet and prevention of coronary heart diseases (Visioli & Galli 1994).

Effects on the cardiovascular system

Antihypertensive activity

O. europaea extracts also appear to have some interesting effects on the cardiovascular system that are unrelated to their antioxidant properties , including blood pressure lowering and anti-arrhythmic actions, and effects on coronary blood flow in certain situations.

O. europaea extract of fresh leaves administered to rats in a single dose of 360 mg/kg daily showed spasmolytic activity against phenylephrine-induced contractions (in 5 minutes 40% and in 15 minutes 25%) (Lasserre *et al*. 1983; Blaschek *et al.* 2006).

European olive leaf and shoot has been administered in the rat intragastrically (i.g.) at doses of 25 mg/kg, following aconite-induced arrhythmia. In the same study, antihypertensive activity was demonstrated by glycerine: ethanol (50:50) extracts given i.g. to the rat at high dosages of 125-250 mg/kg, following desoxycorticosterone acetate (DCSA)-induced hypertension. Positive inotropic effects of 95% ethanol, glycerine and ethanol: glycerine (50:50) extracts were demonstrated in the rabbit at dosages of 5 mg/ml (heart). Spasmolytic activity of similar extracts was demonstrated in the guinea pig at doses of 50 mg/kg against vasopressin-induced coronary spasm and hypotensive activity in the rat at doses of 100 mg/kg (i.g). Maximum hypotensive activity effect was seen 60-120 minutes after administration of each extract. Positive chronotropic effects of glycerine: ethanol (50:50) extracts were noted, when given i.g., to the DCSA-hypertensive rabbit at a dose of 125 mg/ml (Circosta *et al*. 1990).

The effects of a glyceroethanolic macerate of the leaves of *O. europaea* L. and of oleuropein on excito-conduction and on the right atrial and ventricular monophasic action potential have been studied in anaesthetised dogs using the technique of endocavitary recording. At the higher doses tested a slight increase in the sinusal cycle of the sinoatrial conduction time and of the sinus node recovery time, together with a prolongation of the atrioventricular and intraventricular conduction and an increase of the atrial and ventricular monophasic action potential duration were observed. This may be due to a decrease in the repolarisation phase 3. These electrophysiological effects indicate an inhibitory action both on the swift influx of sodium and on the slow influx of calcium, as well as a decrease in potassium conductance (Occhiuto *et al*. 1990).

Leaf decoctions or lyophilised extracts of fresh olive leaves (25-50 mg/kg, i.v.) administered to the rat (aorta) showed spasmolytic activity against phenylephrine-induced contractions, both in the presence of and without endothelium (IC_{50} 1.12 mg/ml) (Zarzuelo *et al*. 1991). Spasmolytic activity of dried leaf extracts (30% ethanol) has been demonstrated, when administered (aorta) to the rabbit in doses of 1 mg/ml (Rauwald *et al*. 1994).

Some of the cardio-vascular effects noted for *O. europaea* have been attributed to the secoiridoids oleuropein (increased coronary flow) and oleacein (ACE inhibitory activity) (Hansen *et al*. 1996).

A special prepared olive leaf extract (EFLA 943) has been tested for its blood pressure lowering activity in rats rendered hypertensive by daily oral doses of L-NAME (NG-nitro-L-arginine methyl ester, 50 mg/kg) for at least 4 weeks. Oral administration of the extract at different dose levels at the same time as L–NAME for a period of 8 weeks showed a dose dependent prophylactic effect against the rise in blood pressure induced by L–NAME, best effect being induced by a dose of 100 mg/kg of the extract. In rats previously rendered hypertensive by L-NAME for 6 weeks and then treated with that dose of the extract for a further 6 weeks without discontinuation of L-NAME, normalisation of the blood pressure was observed. The findings confirm previous reports on the hypotensive effects of olive leaf. The special extract, has shown to give consistent results with little individual variability. The antihypertensive effect of the extract (EFLA 943) maybe related to a variety of factors involving reversal of vascular changes involved in the L-NAME (Khayyal *et al*. 2002).

Effects of a commercial *O. europaea* leaf extract (OLE, not further specified) on isolated hearts and cultured cardiomyocytes have been investigated. Isolated rabbit hearts were perfused according to the Langendorff technique and connected to a 256-channel epicardial mapping system. Voltage clamp experiments were performed in cultured neonatal rat cardiomyocytes using a perforated-patch technique. Results: caused a concentration-depended decrease in systolic left ventricular pressure and heart rate as well as an increase in relative coronary flow and a slight, but not significant prolongation of PQ-time. There were no significant changes between the groups in the activation-recovery interval and its dispersion, total activation time, peak-to-peak amplitude, percentage of identical breakthrough-points and similar vectors of local activation. Voltage clamp experiments in cultured neonatal rat cardiomyocytes showed a significant decrease in maximum ICa, L by OLE which was reversible upon wash-out. Conclusions: OLE suppresses the L-type calcium channel (ICa, L) directly and reversibly. These findings might help to understand the traditional use of OLE in the treatment of cardiovascular disease (Schefflera *et al*. 2008).

Antihyperglycaemic / Hypoglycaemic activity

An early study, using ethanol leaf extracts (defatted with petrol ether) given by gastric intubation to the rabbit (dose not specified), showed a 17-23% decrease in blood sugar levels which reached a minimum within 6 hours and rose to normal after 48 hours (Manceau *et al*. 1942).

Aqueous decoctions of Spanish olive leaf, administered i.g. to the rat at a dose of 32 mg/kg, showed hypoglycaemic activity against alloxan-induced hyperglycaemia. Maximum hypoglycaemic activity was obtained from samples collected in the winter months, especially in February. One of the compounds responsible for this activity was oleuropeoside, which showed activity at a dose of 16 mg/kg. This compound also demonstrated antidiabetic activity in animals with alloxan-induced diabetes. The hypoglycaemic activity of this compound may result from two mechanisms:
(a) potentiation of glucose-induced insulin release, and (b) increased peripheral uptake of glucose (Gonzalez *et al*. 1992).

Aqueous extracts of dried olive leaves from Italy, administered i.g. to male rats in a very high dosage of 500 mg/kg, reduced the blood glucose levels of normal or alloxan-induced diabetic rats (Trovato *et al*. 1993).

The hypoglycaemic activity of olive leaf has been demonstrated in animals. In one study the significance of supplementation of oleuropein in reducing oxidative stress and hyperglycaemia in alloxan-induced diabetic rabbits has been evaluated. In rabbits with induced diabetes, an ethanol extract (75% ethanol) of olive leaf decreased blood glucose as well. Suggested mechanisms include potentiation of glucose-induced insulin release and increased peripheral uptake of glucose. (Al-Azzawie & Alhamdani 2006; Gonzalez *et al*. 1992). Dried leaf powder extracts of Egyptian olive trees collections, when administered intragastrically to the rat, in a dose of 750 mg/kg, were found to be inactive in streptozotocin-induced hyperglycaemia (Eskander & Jun 1995).

Studies in laboratory animals have reported mainly hypoglycaemic activity of olive leaves (Bennani-Kabchi *et al*. 1999; Gonzalez *et al*. 1992). The active constituent was reported to be oleuropein, with a potentiation of glucose-induced insulin release as proposed mechanism of action, as well as an increase in peripheral blood glucose uptake. Especially, in the study of Bennani-Kabchi *et al*. 1999, sand rats develop obesity, insulin resistance, hyperlipidaemia and prediabetes, when given a standard laboratory chow diet. This model has been used to demonstrate the beneficial action of *O. europaea* var. *oleaster* leaves to regulate unbalanced metabolism. Thirty-two sand rats fed on hypercaloric diet during 7 months, were divided into 3 groups: controls (n = 10), treated with the plant (n = 13) and treated with the hypocholesterolemic drug simvastatin. The plant decoction prepared at 10% was given orally at the rate of 1.5 ml/100 g during 3 months. Results show that the plant presents a

hypocholesterolemic effect (42%) related to decreases in LDL and VLDL cholesterol. In addition, hypoglycaemic (16%) and antihyperglycemic (40%) effects were observed accompanied by a 27% decrease in insulin. Chronic treatment reduced total cholesterol (32%), LDL and VLDL cholesterol. Both treatments produced no significant reduction in plasma levels of triglycerides and HDL cholesterol. No toxic effects of this plant have been observed in usual doses (Bennani-Kabchi *et al*. 1999).

In another experimental model of diabetes, induced by streptozotocin, olive leaf failed to lower blood glucose levels or prevent glucosuria and ketonuria but it did not reduce circulating levels of liver enzymes and minimised histopathologic abnormalities in both the kidneys and liver (Onderoglu *et al*. 1999).

Thyroid activity

Lyophilised extracts of freeze dried Saudi Arabian leaf samples, proved active *in vivo* in the male rat. Given intragastrically in doses of 500, 250 and 100 mcg/animal, to rats for 14 days increased triiodothyronine (T3) levels and reduced circulating thyroid-stimulating hormone (TSH) levels, possibly via a feedback mechanism no increase in thyroxine level, a decreased T3 level and TSH inhibition was recorded respectively (Al Qarawi *et al*. 2002).

Anti-complement effects

Anti-complementary activity appears to reside in several flavonoids present in olive leaf extracts. Reported anti-complement *in vitro* activity of olive leaf is a proposed mechanism of its anti-inflammatory effects. From an extract of olive leaf (*O. europaea* L.) showing anti-complementary activity, the flavonoids apigenin, apigenin-4'-O-rhamnosylglucoside, apigenin-7-O-glucoside, luteolin, luteolin-4'-O-glucoside, luteolin-7-O-glucoside, chrysoeriol, chrysoeriol-7-O-glucoside and quercetin-3-O-rhamnoside were isolated. Major isolated constituents strongly inhibited the classical pathway of the complement system (Pieroni *et al*. 1996; PDR for Herbal Medicines, 2007).

Smooth muscle relaxant effects

In experiments demonstrating that a dried extract of olive leaf has relaxant effects on both isolated rat ileal tissue and rat tracheal segments, the effects were not altered in the presence of calcium antagonists including verapamil and nifedipine. It is possible however, that olive leaf extract alters calcium transport though an increase in the intracellular concentration of cyclic adenomonophosphate (Fehri *et al*. 1995)

Renal effects

Ethanol: water (50:50) extracts of fresh olive leaf from Brazil, administered perorally to the rats in doses of 40 ml/kg, showed diuretic activity (Ribeiro *et al*. 1986).

Effects on the inflammatory response

Aqueous leaf extracts from Tunisia, given intragastrically to the rat (dose unspecified), showed activity against carrageenan-induced paw oedema (Fehri *et al*. 1996).

Hepatic activity

In vivo glutathione S-transferase induction activity has been demonstrated in mice given olive leaf extracts in the diet (ethyl acetate extract: 0.4% of diet; methanol extract: 1% of diet) (Han *et al*. 2001).

SECONDARY PHARMACODYNAMICS

Some of the cardio-vascular, antimicrobial, antioxidative, hypoglycaemic effects noted for *O. europaea* have been attributed to the secoiridoids (mainly oleuropein), phenolic compounds as well as triterpenes derived from the leaves, fruits and oil of the olive tree which have been shown through the following *in vitro* and *in vivo* studies to possess biological properties.

In vitro tests

Flavonoids

Antioxidant activity

A study was done to identify the major phenolic compounds present in an extract of olive leaf and estimate their antioxidant activity by their ability to scavenge the radical cation ABTS. Several structural attributes of flavonoids present in olive leaf, including 3-hydroxyl groups, influenced the ability of these compounds to scavenge free radicals. Radical scavenging capacity increased with the number of free hydroxyl groups present in the flavonoid structure. The flavonoid rhamnoglucoside rutin was the most effective compound. The flavonoids, oleuropeosides and substituted phenols present in olive leaf extract exhibited synergism with respect to antioxidant activity (Benavente-García *et al*. 2000). Caffeic acid was also reported to have antioxidant activity through the scavenging of superoxide anion (Chimi *et al*. 1991, 1995).

Olive leaf contains flavonoids that possess antioxidant activity, and tissue antioxidant status has been proposed as a key factor in the development of diabetic complications. This may help explaining why an orally administrated preparation of olive leaf substantially diminished tissue damage in the kidney and liver in rats with streptozotocin induced diabetes (Onderoglu *et al*. 1999).

Flavonoids (luteolin-7-O-β-glucoside and luteolin-4'-O-β-glucoside from olive leaf extracts have shown anti-α-amylase activity from human saliva and pancreas with IC_{50} values of 0.5 and 0.3 mg/ml, respectively (Komaki *et al*. 2003) which is in accordance with previous reported results on luteolin (Kim *et al*. 2000).

Caffeic acid, luteolin and luteolin-7-O-β-glucoside from an ethanolic extract of olive leaf, in a very recent study (Fleming *et al.* 2011) showed a strong inhibition of xanthine oxidase, an enzyme well known to contribute significantly to the pathological process of gout.

Oleuropein

Antioxidant activity

Phenolic compounds derived from the leaves, fruits and oil of the olive tree (*O. europaea* L.) have long been known to have anti-oxidative properties (Chimi *et al*. 1991; Sheabar & Neeman 1988; Petroni *et al*. 1995). More recently, LeTutour & Guedon (1992) demonstrated that oleuropein, hydroxytyrosol, and in particular, extracts of *O. europaea* leaf (containing 19% oleuropein, 1.8% flavonoid glycosides, and 3,4-dihydroxy- phenethyl esters) were more potent antioxidants than vitamin E or butylhydroxytoluene (BHT), in a model chemical system (inhibition of oxidation of methyl linoleate in heptanol or propanol-water, initiated by 2,2'-azo-bis-isobutyronitrile). Another *in vitro* study (Visioli & Galli 1994) showed that oleuropein (at a concentration of 10^{-5} M) significantly inhibited copper sulphate-induced oxidation of LDL extracted from normal human plasma.

Oleuropein and hydroxytyrosol, two phenolic compounds contained in olives and olive oil, are known to possess several biological properties, many of which may be related, partially at least, to their

antioxidant and free radical-scavenger ability. Hence, together with their scavenging activity against the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH test), the antioxidative effect of oleuropein and hydroxytyrosol were investigated in a model system consisting of dipalmitoylphosphatidylcholine / linoleic acid unilamellar vesicles (DPPC/LA LUVs) and a water-soluble azo compound as a free radical generator (LP-LUV test). The results obtained were also interpreted in the light of biophenol interactions, studied by differential scanning calorimetry, with dimyristoylphosphatidylcholine (DMPC) vesicles as a biological membrane model. The results obtained in the DPPH and LP-LUV tests confirm the good scavenger activity and antioxidant effect of oleuropein and hydroxytyrosol. However, while both compounds exhibit comparable effectiveness in the DPPH test (hydroxytyrosol being slightly more active than oleuropein), oleuropein seems, in the LP-LUV test, a better antioxidant than hydroxytyrosol. Besides oleuropein shows a better antioxidant activity in the membranous system than in homogenous solution. Furthermore, oleuropein, but not hydroxytyrosol, interacts with DMPC vesicles, causing shifts, toward lower values, of the calorimetric peak temperature , associated to the gel to liquid- crystal phase transition, typical for DMPC multilayers. The authors hypothesized that hydroxytyrosol can serve as scavenger of aqueous peroxyl radicals near the membrane surface, while oleuropein acts also as a scavenger of chain-propagating lipid peroxyl radicals within the membranes (Saija *et al*. 1998).

Cardiovascular effects

Studies have shown that oleuropein possesses a wide range of pharmacologic and health promoting properties including antiarrhythmic, spasmolytic, immune-stimulant, cardio protective, hypotensive, and anti-inflammatory effects (Petkov & Manolov 1978; Visioli *et al*. 1995, 1998ii; Circosta *et al*. 1990; Diaz *et al*. 2000; Somova *et al*. 2004). Many of these properties have been suggested as a result from the antioxidant character of oleuropein (Visioli *et al*. 2002). Most of the reported antioxidant characteristics of oleuropein are drawn from *in vitro* investigations (Amro *et al*. 2002; Stupans *et al*. 2002; Ferroni *et al*. 2004), and even those who involved animals or human subjects the antioxidant activity of oleuropein was demonstrated in a condition at which there is no established oxidative challenge (Visioli *et al*. 2000).

Olive leaf has antioxidant properties associated with phenolic constituents and oleuropein (Turner *et al*. 2005). Oleuropein, an antioxidant has been reported to decrease the oxidation of LDL cholesterol (Visioli *et al*. 1994). Oxidized LDL is the most damaging form of cholesterol and can initiate damage to arterial tissues, thereby promoting atherosclerosis.

The effects of oleuropein were studied on the electromechanical properties of isolated guinea-pig atria. In spontaneously beating right atria, oleuropein decreased the amplitude of contractions (IC_{50} = 1.3 ± 0.2 x 10^{-4} M), slightly decreased atrial rate, and lengthened sinus node recovery time and also inhibited peak contractile force in electrically driven left atria incubated in normal (IC_{50} = 1.5 ± 0.5 x 10^{-4} M) and in 27 mM K^+ Tyrode solution (IC_{50} = 1.7 ± 0.4 x 10^{-4} M). These negative inotropic effects were not accompanied by significant changes in the characteristics of action potentials recorded in atria incubated in either normal or depolarising solutions. These results indicate that oleuropein produced an electromechanical uncoupling that cannot be attributed to an inhibitory effect on Ca^{2+} entry through L-type channels (Duarte *et al*. 1993).

Antimicrobial activities

A variety of antibacterial actions of oleuropein and its associated compounds have been demonstrated *in vitro*.

The component usually associated with olive leaf's antimicrobial properties is oleuropein (Petkov & Manolov 1972; Juven & Henys 1972).

Fleming *et al* (1973) isolated six major phenolic compounds from green olives; one particular compound, possibly a hydrolysis product of oleuropein, was more active than oleuropein itself against the lactic acid bacterium *Leuconostoc mesenteroides* FBB 42. Later on, the oleuropein aglycone and elenolic acid were found to strongly inhibit the growth of three further species of lactic acid bacteria – *Lactobacillus plantarum, Pediococcus cerevisiae* and *Lactobacillus brevis* (Fleming *et al*. 1973). Since the aglycone is composed of elenolic acid bound to b-3,4-dihydroxyphenylethyl alcohol and the latter compound was not inhibitory, the investigators concluded that elenolic acid was the inhibitory part of the aglycone molecule. Oleuropein itself was not inhibitory to these bacteria, but did inhibit three species of non-lactic acid bacteria – *Staphylococcus aureus, Bacillis subtilis* and *Pseudomonas solanecearum*. In addition, an acid hydrolysate of an extract of oleuropein (containing hydrolysis products of oleuropein not specifically identified) inhibited the growth of a further eight species of bacteria. Later *in vitro* studies have shown that oleuropein and/or its hydrolysis products also inhibit the germination and sporulation of *Bacillus megaterium* (Rodriguez *et al*. 1988) and inhibit outgrowth of germinating spores of *Bacillus cereus* T. (Tassou *et al*. 1991).

Oleuropein has also been reported to directly stimulate macrophage activation in laboratory studies (Visioli *et al*. 1998i). Besides, oleuropein has shown anti-microbial activity against yeasts, fungi, molds and other parasites (Aziz *et al*. 1998; Koutsoumanis *et al*. 1998). Hydroxytyrosol demonstrated broader antimicrobial activity than oleuropein and is comparable to ampicillin and erythromycin in spectrum and potency (Bisignano *et al*. 1999; Khan *et al*. 2007).

The activity of oleuropein, a phenolic glycoside contained in olive oil, was investigated *in vitro* against *Mycoplasma hominis, Mycoplasma fermentans, Mycoplasma pneumoniae* and *Mycoplasma pirum*. Oleuropein inhibited mycoplasmas at concentrations from 20 to 320 mg/l. The MICs of oleuropein to *Mycoplasma pneumoniae, Mycoplasma pirum, Mycoplasma hominis* and *Mycoplasma. fermentans* were 160, 320, 20 and 20 mg/l, respectively (Furneri *et al*. 2002).

Other activities

In vitro studies have demonstrated that oleuropein acts as an anti-tumour compound (Saenz *et al*. 1998), inhibits platelet-activating factor activity (Andrikopoulos *et al*. 2002), enhances nitric oxide production by mouse macrophages (Visioli *et al*. 1998i) and decreases inflammatory mediator production (Miles *et al*. 2005).

The antioxidant/anticancer potential of phenolic compounds isolated from olive tree (Owen *et al*. 2000), as well as the *in vitro* cytotoxicity to human cells in culture of some phenolics from olive oil, has been reported by Babich & Visioli (2003) as well as by Hamdi & Castellon (2005); who have shown the activities of oleuropein, as an anti-tumour agent and cytoskeleton disruptor.

Besides oleuropein exhibits proteasome stimulatory properties *in vitro* and confers life span extension of human embryonic fibroblasts (Katsiki *et al*. 2007).

Elenolic acid

Antiviral activity

In addition to its antibacterial actions, elenolic acid has been shown to be a potent inhibitor of a wide spectrum of viruses. Olive leaf extract has reported antiviral activity, caused by the constituent calcium elenolate, a derivative of elenolic acid (Renis 1970; Heinze *et al*. 1975). The isolated calcium salt of elenolic acid was tested as a broad-spectrum antiviral agent active against all viruses tested (Soret 1969). Some viruses inhibited by calcium elanolate *in vitro* include rhinovirus, myxoviruses, Herpes simplex type I, Herpes simplex type II, Herpes zoster, Encephalomyocar ditis, Polio 1, 2, and 3, two strains of leukemia virus, many strains of influenza and para-influenza viruses vaccinia, pseudorabies,

influenza A (PR8), Newcastle disease, parainfluenza 3, Coxsackie A21, encephalomyocarditis, polio 1, 2 and 3, vesicular stomatitis, Sindbis and reovirus 3 (Deering) viruses (Renis 1975; Soret 1969; Hirschman 1972). Calcium elenolate also inhibits the RNA-dependent DNA polymerase I enzymes (reverse transcriptases) of murine leukaemia viruses (MuLV(M), (Hirschman 1972) and the DNA polymerase II and III enzymes of Eschericha coli (Heinze *et al*. 1975) *in vitro*.

The mechanism of action of the antiviral activity is reported to include:

i) ability to interfere with critical amino acid production essential for viruses

ii) ability to contain viral infection and/or spread by inactivating viruses or by preventing virus shedding, budding, or assembly at the cell membrane

iii) ability to directly penetrate infected cells and stop viral replication

iv) in the case of retroviruses, it is able to neutralise the production of reverse transcriptase and protease

v) stimulation of phagocytosis

Triterpenes

Hypoglycaemic effect of α-amylase

The triterpene oleanolic acid on the activities of human amylases was examined *in vitro*. It has inhibited the activities of α-amylase from human saliva and pancreas with IC_{50} value of 0.1 mg/ml (Komaki *et al*. 2003).

In vivo tests

Oleuropein

Antioxidant activity

Rabbits with induced diabetes showed a decrease in oxidative stress markers when treated with oleuropein (Al-Azzawie & Alhamdani 2006). Other experiments support the antioxidant activity of the phenols oleuropein and hydroxytyrosol (Benavente-Garcia *et al*. 2000; Briante *et al*. 2001, 2002; Visioli *et al*. 2002; Caturla *et al*. 2005).

Cardiovascular effects

Petkov and Manolov (1972) observed in their investigations of the cardiovascular effects of oleuropein in animals that 3-50 mg/kg oleuropein given i.p. caused a slight stimulation of the respiratory rate in anaesthetised cats. In dogs with experimentally induced hypertension, 10-30 mg/kg oleuropein caused a sharp, long-lasting drop in both systolic and diastolic blood pressure in three out of four animals, and a lesser, shorter-lived decrease in blood pressure in the fourth dog. The same investigators found that oleuropein caused an increase in blood flow through the coronary vessels of isolated rabbit heart preparations, but no change in coronary flow in anaesthetised cats at doses of 10-30 mg/kg. However, in a model of experimentally disturbed coronary circulation, oleuropein (30 mg/kg intravenously) largely abolished the characteristic ECG (electrocardiogram) changes caused by Pituitrin (which diminishes coronary blood flow) in conscious rabbits, when given 1 minute after the Pituitrin injection. Lastly, found that oleuropein eliminated cardiac arrhythmia in dogs with induced hypertension for 1.5-2 hours, normalised cardiac rhythm in rabbits with barium chloride-induced arrhythmia for about 1 hour, and prevented or reduced the duration of disturbed cardiac rhythm in rats

with calcium chloride-induced arrhythmia. The pharmacological mechanisms underlying any of these effects on the heart and vasculature are unknown. Also, in doses of 10-30 mg/kg, it caused a brief depressed state with decreased motor activity in two out of four conscious dogs with induced hypertension, and was badly tolerated in a third dog, causing excitation, scratching, and vigorous jolting movements, red, watery eyes, and hyperaemic (warm, reddened) abdominal skin.
Some of the cardio-vascular effects noted for *O. europaea* have been attributed to the secoiridoids oleuropein increased coronary flow and oleacein (ACE inhibitory activity) (Hansen *et al*. 1996).

Ruiz-Gutierrez *et al*. (2000) investigated the effects of oleuropein on lipids and fatty acids in heart tissue, did not report any adverse behavioural or other effects (for example, on food consumption, body weight, heart weight or heart total lipid content) in rats given intraperitoneal injections of 25 or 50 mg/kg daily for 3 weeks. Oleuropein did significantly reduce the linoleic acid content and the ratio of unsaturated to saturated fatty acids in heart polar lipids, depleted heart levels of vitamin E and itself became incorporated in heart tissue, but the significance of these findings is unclear. However, heart tissue that had been pre-treated with oleuropein *in vitro* was not susceptible to peroxidation.

In vivo, studies in rats indicate that oleuropein prevents oxidative myocardial injury (Manna *et al*. 2004).

Herbal preparations in animal experiments in rabbit and rats found a hypotensive effect of oleuropein, possibly via direct action on smooth muscle. Oleuropeoside also may exert a vasodilatory activity.

Finally, the anti-ischemic, anti-oxidative and hypolipidemic effects of oleuropein in anesthetised rabbits were recently evaluated. It has been seen that the plasma lipid peroxidation products and carbionyl concentrations compared with the control groups in which these factors increased relative to baseline due to ischaemia and reperfusion. Treatment for 6 weeks with both doses of oleuropein (10 and 20 mg/ml) reduced total cholesterol and triglycerides concentrations. This is the first experimental study *in vivo* that suggests the possibility of using oleuropein in the treatment of ischemia (Andreadou *et al*. 2006).

Anti-hyperglycaemic activity

Oleuropein is reported to have an anti-hyperglycaemic effect in diabetic rats (Gonzalez *et al*. 1992). However, regarding the antioxidant feature of oleuropein, it is still unknown if oleuropein may exert other beneficial effects in diabetes as in attenuating oxidative stress.

Patients with diabetes mellitus are likely to develop certain complication such as retinopathy, nephropathy and neuropathy as a result of oxidative stress and overwhelming free radicals. Treatment of diabetic patients with antioxidant may be of advantage in attenuating these complications. One study aimed to evaluate the significance of supplementation of oleuropein in reducing oxidative stress and hyperglycaemia in alloxan-induced diabetic rabbits. After induction of diabetes, a significant rise in plasma and erythrocyte malondialdehyde (MDA) and blood glucose as well as alteration in enzymatic and non-enzymatic antioxidants was observed in all diabetic animals. During 16 weeks of treatment of diabetic rabbits with 20 mg/kg body weight of oleuropein the levels of MDA along with blood glucose and most of the enzymatic and non-enzymatic antioxidants were significantly restored to establish values that were not different from normal control rabbits. Untreated diabetic rabbits on the other hand demonstrated persistent alterations in the oxidative stress marker MDA, blood glucose and the antioxidant parameters. The authors conclude that oleuropein may be of advantage in inhibiting hyperglycaemia and oxidative stress induced by diabetes and suggest that administration of oleuropein may be helpful in the prevention of diabetic complications associated with oxidative stress (Al-Azzawie & Alhamdani 2006).

Other activities

Other clinical effects of oleuropein are the potentiation of cellular and organismal protection through the macrophage-mediated response (Visioli *et al*. 1998i) and the inhibition of platelet aggregation and eicosanoid production (Petroni *et al*. 1995). Olive oil and its main phenolic constituent (oleuropein) prevent inflammation-induced bone loss in the ovariectomised rat (Puel *et al*. 2004).

Natalini and co-workers have postulated that oleuropein might be a modulator of metabolism via pepsin activation and inhibition of others enzymes such as trypsin. (Polzonetti *et al*. 2004).

The molluscicidal (anti-schistosomial) activity of oleuropein against the mollusc *Biomphalaria glabrata* has been reported showing an LD_{50} 250 ppm within 24 hours (Kubo & Matsumoto 1984; Blaschek *et al.* 2006).

Oleuropein has been patented in the United States for antiviral activity against viral diseases, including herpes, mononucleosis, and hepatitis (Fredrickson 2000).

Calcium elenolate

Antiviral activity

Soret (1969) showed that calcium elenolate effectively reduced viral titres *in vivo* when given before and/or after inoculation of hamsters with myxovirus parainfluenza type 3 (HA-1 virus, strain C-243). Treatment with calcium elenolate, but not placebo, prevented spread of viral infection to the lungs.

Triterpenes

Cardiovascular effects

A bioassay guided study of triterpenoids isolated from the leaves of *O. europaea* from Greece, from wild African olive and from cultivar of *O. europaea* grown in Cape Town was reported. The experiment was undertaken since the preliminary analyses showed that the African wild olive leaves are rich in triterpenoids and contain only traces of oleuropein which is typical for European olive leaves. The anti-hypertensive, diuretic, anti-atherosclerotic, antioxidant and hypoglycaemic effects of authentic oleanolic and ursolic acid and the three isolates were studied on Dahm-salt –sensitive insulin resistant rat genetic model of hypertension. All the three isolates in a dose 60 mg/kg body weight for 6 weeks treatment prevented the development of severe hypertension and atherosclerosis and improved the insulin resistance of the experimental animals (Somova *et al*. 2002; 2003). The same derivatives have acted as beta-adrenergic antagonists, blocking the effect of adrenaline and isoprenaline. The authors are of the opinion that the three triterpenes could provide an effective and cheap and accessible source of additive to conventional treatment of hypertension, complicated by stenocardia and cardiac failure in the African population (Somova *et al*. 2004).

SAFETY PHARMACOLOGY

Laboratory experiments evaluating safety pharmacology were not fully performed. Therefore, safety parameters and the benefit-risk ratio must be derived from general toxicological properties of the components and the traditional use of olive leaf extracts.

PHARMACODYNAMIC INTERACTIONS

Oleuropein is among the herbal constituents that act as mechanism-based inhibitors of various Cytochrome P450 enzymes (CYPs) (like capsaicin from chilli, glabridin from liquorice, resveratrol in red wine etc.). This may provide explanation for some reported herb-drug interactions. In addition, the

inhibition of CYPs by herbal constituents may decrease the formation of toxic metabolites and thus inhibit carcinogenesis, as CYPs play an important role in procarcinogen activation (Zhou *et al*. 2007).

Pharmacodynamic drug interactions of whole extracts or isolated constituents have not been reported.

ASSESSOR'S OVERALL CONCLUSIONS ON PHARMACOLOGY

Olive leaf as herbal substance and/or herbal preparation has antihypertensive, hypolipidemic and diuretic activities, mainly due to its secoiridoids constituents (oleuropein) as well as phenolic constituents (especially flavonoids). Together with their strong antioxidant activities, which contribute to resist oxidation, a supporting action to the cardiovascular system and function is assumed. Other possible pharmacodynamic actions including hypoglycaemic (in high doses), antimicrobial, antiviral, hepatic, smooth muscle relaxant as well as effects on the inflammatory response. Taken together such bioactivities help to account for some of the existing clinical effects.

3.2. Overview of available pharmacokinetic data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

Herbal substance / Herbal Preparations

No data on *Olea* extracts have been found or reported, while there is only the following reference on oleuropein purified from *Olea* extracts.

Oleuropein

There are insufficient data in the literature to fully understand the bioavailability of polyphenols such as oleuropein, hydroxytyrosol and tyrosol. It is known that oleuropein is poorly absorbed due to its large size and planar configuration (Edgecombe *et al*. 2000). It is however hypothesised that since oleuropein is a glucoside, it could probably access a glucose transporter (SGLT1) found on the epithelial cells of the small intestine, permitting its entry into the cells. Conversely, it was postulated in previous investigations that the absorption of the quercetin glycoside (a similar polyphenolic) involved active sugar transporters (Singh *et al*. 2008).

Other studies have shown that oleuropein is rapidly absorbed after oral administration, with maximum plasma concentration occurring 2 hours after administration. Hydroxytyrosol was its most important metabolite. Both compounds are rapidly distributed and excreted in urine as glucoronides or in very low concentrations as free forms (Tan *et al*. 2003; Boccio *et al*. 2003; Vissers *et al*. 2002).

Assessor's overall conclusions on pharmacokinetics

Limited data are available on pharmacokinetics. No data are available for the herbal substance or the herbal preparation and therefore no conclusion can be drawn. Only some data exist for oleuropein and its metabolites. Oleuropein is also among the herbal constituents which behave as mechanism-based inhibitors of various CYPs.

3.3. Overview of available toxicological data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof

Olive leaf

Single dose toxicity

The LD_{50} of an extract (not specified) of olive leaf (*O. europaea*) was given 1300 mg/kg, i.p. in mouse; > 3000 mg/kg orally in mouse (Duke 2002; Blaschek *et al.* 2006), besides at 1 mg/ml, an extract of olive leaf was not toxic to human cells (Lee-Huang *et al*. 2003).

Chronic oral toxicity

No information on olive leaf are available.

Oleuropein

Petkov and Manolov (1972) gave single daily intraperitoneal doses of oleuropein to albino mice ranging from 100 to 1000 mg/kg (in solutions of 1, 5 and 10%). No toxic effects or deaths during the 7-days post-treatment period were observed, and consequently oleuropein's LD_{50} could not be determined in this study (Petkov & Manolov 1972; Blaschek *et al.* 2006).

Calcium elenolate

Acute toxicity

Elliott *et al*. (1969) determined the LD_{50} for calcium elenolate to be 120 mg/kg in mice when given intraperitoneally, and 160 mg/kg in rats via the intraperitoneal route and 1,700 mg/kg via the oral route.

Repeated dose toxicity

Elliott *et al*. (1969) found calcium elenolate to be well tolerated in rats given daily oral doses of 0, 30, 100 or 300 mg/kg for 1 month. The only drug-related change observed was a yellowing of the non-glandular fore-stomach in 40% of the rats receiving the highest dose (300 mg/kg). In 7-month-old beagle dogs given daily oral doses of 0, 3, 10 or 30 mg/kg calcium elenolate for 1 month, all but the highest dose were well tolerated – three out of the four dogs receiving 30 mg/kg showed a mild gastric irritation with sporadic vomiting. Tissue analysis revealed a few small gastric erosions in these animals.

Blood toxicity

No blood toxicity studies have been carried out according to available scientific literature.

Genotoxicity

No genotoxicity studies have been carried out according to available scientific literature.

Carcinogenicity

No carcinogenicity studies have been carried out according to available scientific literature.

Teratogenicity

No teratogenicity studies have been carried out according to available scientific literature.

Immunotoxicity

No immunotoxicity studies have been carried out according to available scientific literature.

Assessor's overall conclusions on toxicology

There are only limited preclinical safety data for olive leaf extracts and some limited toxicological data concerning the toxicity of oleuropein and calcium elanolate mainly published in the 70's, considered to be insufficient.

Due to the lack of data on mutagenicity, carcinogenicity and reproductive and developmental toxicity, a list entry for Oleae folium cannot be recommended.

3.4. Overall conclusions on non-clinical data

Olive leaf was officially used in Germany as a herbal remedy traditionally used to support cardiovascular system while in France is used for elimination functions and to help digestion. Moreover, Olive leaf is used in Spain and other European countries as a traditional remedy for more than 30 years without safety problems.

The published data with respect to the indications and preparations is limited. On the basis of existing pharmacological data mainly on *Olea* constituents antihypertensive, hypolipedimic and diuretic, antioxidant activities are reported. Furthermore hypoglycaemic (in high doses), antimicrobial, antiviral, smooth muscle relaxant as well as effects on the inflammatory response were described.

Some of these data support the traditional use of *O. europaea* and preparations thereof in the proposed indication:
Traditional herbal medicinal product used to promote the renal elimination of water, in mild cases of water retention.

The efficacy of traditional herbal medicinal products is only plausible but not proven by clinical data. The lack of genotoxicity, carcinogenicity as well as reproductive and developmental toxicity studies do not allow the establishment of a Community List Entry.

4. Clinical Data

4.1. Clinical Pharmacology

Preclinical studies have shown that olive leaf extracts and therein contained phenolic compounds as well as secoiridoids as oleuropein protect the cardiovascular system mainly through their antioxidant activity.

4.1.1. Overview of pharmacodynamic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

The flavonoid polyphenols in olive leaves are natural antioxidants that have a host of health beneficial effects (Visioli *et al.* 1998i). The active phenolic compounds in the olive leaf extract are part of the secoiridoid family, known for their capacity to scavenge H_2O_2. Pignatelli *et al*. demonstrated that following stimulation by collagen, there is a burst of hydrogen peroxide in the process of platelet activation. H_2O_2 activates the enzyme phospholipase C, which brings about arachidonic acid metabolism and platelet aggregation (Singh *et al*. 2008).

Previous studies demonstrated that oleuropein and hydroxytyrosol due to their capacity to scavenge H_2O_2, inhibited the respiratory burst of human neutrophils elicited by phorbol 12-myristate 13-acetate in a dose dependant fashion (Visioli *et al*. 1998ii).

Several polyphenols have been found in the olive leaves even though oleuropein was found to be in higher concentration. Other polyphenols like hydroxytyrosol, caffeic acid, luteolin and rutin as well as flavanol cathechin have been also determined (Benavente-Garcia *et al*. 2000). All these phenolics are also established to have antioxidant activity and H_2O_2 scavenging properties. It is believed that there is a synergy to the observed platelet inhibition of all these various polyphenols as opposed to oleuropein alone (Singh *et al*. 2008). Of course further studies will need to be validated with *in vivo* evaluation of platelet activation such as urinary thromboxane B2 excretion and evaluation of oxidative stress markers such as isoprostanes (derived from the non enzymatic peroxidation of arachidonic acid) to provide insight into the mechanism responsible for the inhibition of platelet function by polyphenols.

Hypoglycaemic activity

Flavonoids (luteolin-7-O-β-glucoside and luteolin-4'-O-β-glucoside) from olive leaf extracts were active against α-amylase from human saliva and pancreas with IC_{50} values of 0.5 and 0.3 mg/ml, respectively (Komaki *et al*. 2003) which is in accordance with previous reported results on luteolin (Kim *et al*. 2000). It was reported that oleuropein leaf accelerated the intake of glucose to the cell (Gonzalez *et al*. 1992). An ethanolic extract from olive leaves but not oleuropein itself inhibited α-amylase (Komaki *et al* 1992). Because oleuropein's aglycon moiety inhibited strongly the enzyme (IC_{50} = 0.03 mg/ml) it is suggested that oleuropein also reduces the blood glucose level inhibiting the activity of α-amylase *in vivo*. Furthermore, luteolin-7-O-β-glucoside and luteolin-4'-O-β-glucoside and oleanolic acid isolated from olive leaf ethanolic extract exhibited an inhibitory effect on α-glucosidase prepared from rat intestine *in vitro* (Komaki *et al*. 2003).

Assessor's overall conclusions on pharmacodynamics

At present, the mechanism of action of olive leaf extracts cannot be considered clarified.

4.1.2. Overview of pharmacokinetic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

Phenolic compounds such as oleuropein, phenolic acids and flavonoids are quantitatively important constituents of the whole olive leaf extract. The systemic bioavailability of them is probably relatively low and variable.

Assessor's overall conclusions on pharmacokinetics

Data on pharmacokinetics of Oleae folium extract or relevant components are limited in humans.

4.2. Clinical Efficacy

4.2.1. Dose response studies

No pharmacokinetic or pharmacodynamic studies were performed to support the posology and daily dose proposed.

4.2.2. Clinical studies (case studies and clinical trials)

Antihypertensive effects

Olive leaf extract (with no further details given for the extract used) had an antihypertensive effect in patients with essential arterial hypertension. Patients were separated into two groups: first timers who had never been previously treated with hypotensive medication (n=12) and a second group who had previously benefited from some sort of anti-hypotensive therapy such as diuretic or beta-blocker medication (n=18). For the second group, all therapeutic medications were removed 15 days prior to the beginning of the study. Both groups then received placebo gel capsules for 2 weeks. For the 3 months that followed, the placebo was replaced with similar gel capsules, each containing 400 mg of aqueous olive leaf extract. Patients took 4 capsules daily for total dose of approximately 1.6 g olive leaf extract daily. A significant decrease in blood pressure occurred in all patients ($p < 0.001$). No adverse effects were reported during treatment with olive leaf extract and patients especially noted a disappearance of gastric disturbances that they had previously experienced on beta-blockers medications. As a side note, the authors also found a small but significant decrease of glycaemia ($p < 0.01$) and calcium ($p < 0.001$) in the groups (**Cherif *et al.* 1996**).

A double-blind, randomized, parallel and active-controlled clinical study was conducted to evaluate the anti-hypertensive effect as well as the tolerability of olive leaf extract EFLA®943 in comparison with captopril in patients with stage-1 hypertension (**Susalit *et al.* 2011**).

The extract EFLA®943, manufactured from the dried leaves of *O. europaea* L, is an ethanol (80% m/m) extract. After a patented filtration process (EFLA®Hyperpure), the crude extract was dried. The drug to extract ratio (DER) was 3–7:1. Characteristic components in the extract were 18–26% (m/m) oleuropein, 30–40% (m/m) polyphenols as well as verbascoside and luteolin-7-glucoside (Khayyal *et al.* 2002; Perrinjaquet-Moccetti *et al.* 2008; Susalit *et al.* 2011).

232 patients referred to Nephrology & Hypertension Division, Department of Internal Medicine, of Medicine, University of Indonesia, were enrolled in the study. Of them, 162 (69.8%) subjects completed the study, 16 (6.9%) dropped out from the study due to various reasons and 54 (23.3%) had no available post-treatment data. Among those subjects that completed the study, 14 (6%) were incompliant with respect to study medication (consumption of study medication <80%), resulting in 148 (63.8%) patients evaluable for per-protocol efficacy analysis. Additionally, this study also investigated the hypolipidemic effects of olive leaf extract in such patients. It consisted of a run-in period of 4 weeks continued subsequently by an 8-week treatment period. Olive leaf extract (EFLA®943) was given orally at the dose of 500 mg twice daily in a flat-dose manner throughout the 8 weeks. Captopril was given at the dosage regimen of 12.5 mg twice daily at start. After 2 weeks, if necessary, the dose of captopril would be titrated to 25 mg twice daily, based on subject's response to treatment.

The primary efficacy endpoint was reduction in systolic blood pressure (SBP) from baseline to week-8 of treatment. The secondary efficacy endpoints were SBP as well as diastolic blood pressure (DBP) changes at every time-point evaluation and lipid profile improvement. Evaluation of BP was performed every week for 8 weeks of treatment; while of lipid profile at a 4-week interval. Mean SBP at baseline was 149.3±5.58 mmHg in olive group and 148.4±5.56 mmHg in captopril group; and mean DBPs were 93.9±4.51 and 93.8±4.88 mmHg, respectively.

After 8 weeks of treatment, both groups experienced a significant reduction of SBP as well as DBP from baseline; while such reductions were not significantly different between groups. Means of SBP reduction from baseline to the end of study were −11.5±8.5 and −13.7±7.6 mmHg in olive and captopril groups, respectively; and those of DBP were −4.8±5.5 and −6.4±5.2 mmHg, respectively. A

significant reduction of triglyceride level was observed in the olive group, but not in the captopril group. In conclusion, olive leaf extract, at the dosage regimen of 500 mg twice daily, was similarly effective in lowering systolic and diastolic blood pressures in subjects with stage-1 hypertension as captopril, given at its effective dose of 12.5–25mg twice daily.

A total of 1057 adverse events were reported by 168 (94.4%) study subjects, 83 subjects (49.4%) belonged to the olive group and 85 (50.6%) to the captopril group. The majority of adverse events were tolerably mild (99.8%) and comparable between groups. The most common adverse events which contributed to more than 5% of the total events observed during the study were coughing (4.6% in olive and 7% in captopril group) and vertigo (5.9% in olive and 6.3% in captopril group). Less frequently, muscle discomfort, headache, fatigue, malaise, myalgia and muscle cramp were reported and comparable between groups, constituting less than 5% of the total events. Vertigo, muscle discomfort and headache were judged to be possibly related to both olive leaf extract and captopril. All these adverse events had resolved at the end of the study. Based on the laboratory safety evaluation, it was observed that administration of olive leaf extract to stage-1 hypertensive subjects did not affect liver and renal functions. Neither did it affect haematological parameters and electrolyte balance of study participants. Even though some of the laboratory safety parameters were statistically different from baseline values, all of them remained within the normal range at the end of the study period, and thus such changes were not clinically relevant. The evaluation of all safety parameters and occurrence of adverse events showed that olive leaf extract was safe and tolerable in patients with stage-1 hypertension.

The olive leaf extract EFLA®943, having antihypertensive actions in rats (Khayyal *et al.* 2002), was tested as a food supplement in an open study including 40 borderline hypertensive monozygotic twins. Twins of each pair were assigned to different groups receiving 500 or 1000 mg/day EFLA®943 for 8 weeks, or advice on a favourable lifestyle. Body weight, heart rate, blood pressure, glucose and lipids were measured fortnightly. Blood pressure changed significantly within pairs, depending on the dose, with mean systolic differences of ≤6 mmHg (500 mg vs control) and ≤13 mmHg (1000 vs 500 mg), and diastolic differences of ≤5 mmHg. After 8 weeks, mean blood pressure remained unchanged from baseline in controls (systolic/diastolic: 133 ± 5/77 ± 6 vs 135 ± 11/80 ± 7 mmHg) and the low-dose group (136 ± 7/77 ± 7 vs 133 ± 10/76 ± 7), but had significantly decreased for the high dose group (137 ± 10/80 ± 10 vs 126 ± 9/76 ± 6). Cholesterol levels decreased for all treatments with significant dose dependent within-pair differences for LDL-cholesterol. None of the other parameters showed significant changes or consistent trends. Concluding, the study confirmed the antihypertensive and cholesterol-lowering action of EFLA®943 in humans (**Perrinjaquet-Moccetti *et al.* 2008**).

Renal effects

Diuretic activity was observed in human adult patients given a leaf infusion (5 ml) or decoction (3 ml) by mouth once daily for 20-25 days. A daily increase in urinary output of 100-145 ml was noted for both dosage forms, with no effect on blood Na, K or chloride levels (**Capretti & Bonaconza 1949**).

4.2.3. Clinical studies in special populations (e.g. elderly and children)

No information available.

4.3. Overall conclusions on clinical pharmacology and efficacy

Four existing clinical studies could support the traditional use with a mild diuretic activity as well as antihypertensive activity.

Analytically a very recent double-blind, randomized, parallel and active-controlled clinical study was conducted to evaluate the anti-hypertensive effect as well as the tolerability of olive leaf extract

EFLA®943 in comparison with captopril in 148 patients with stage-1 hypertension (Susalit *et al.* 2011). The evaluation of all safety parameters and occurrence of adverse events showed that olive leaf extract was safe and tolerable in patients with stage-1 hypertension. In another study the same extract (EFLA®943), having antihypertensive actions in rats (Khayyal *et al.* 2002), was tested as a supplement in an open study including 40 borderline hypertensive monozygotic twins. Concluding, the study confirmed the antihypertensive and cholesterol-lowering action of it in the tested humans (Perrinjaquet-Moccetti *et al.* 2008).

According to the published *in vitro* and *in vivo* studies as well as the existing old and not well documented, but also the two very recent clinical trials (Susalit *et al.* 2011; Perrinjaquet-Moccetti *et al.* 2008) a mild diuretic effect as well as a supportive effect to the cardiac system (through decrease of blood pressure) is plausible.

No side effects have been reported during the use of olive leaf preparations.

5. Clinical Safety/Pharmacovigilance

5.1. Overview of toxicological/safety data from clinical trials in humans

The safety profile of olive leaf extracts can be described as acceptable from the limited existing clinical studies and from its use from products on the market. The safety results obtained from the clinical studies conducted so far show that the oral use of olive leaf extracts are well tolerated by most patients. No drug-related serious or moderate adverse events were reported during the existing clinical trial.

5.2. Patient exposure

There are limited data available on the exposure of patients (see also sections 4.1-4.3).

5.3. Adverse events and serious adverse events and deaths

Intra-ocular use of olive leaf may irritate the surface of the eye (PDR for Herbal Medicines 2007). If olive leaf extract preparations are administrated to patients with biliary tract stones, there is a risk of causing biliary colic through promoting the secretion of bile.

Pollinosis, in the form of rhinitis or bronchial asthma has been reported (PDR for Herbal Medicines 2007).

In a recent clinical trial (Susalit *et al.* 2011) with olive leaf extract several adverse events were reported whereof 83 (49.4%) belonged to the olive group. The majority of adverse events were tolerably mild (99.8%) and occurred less frequently than in the captopril group. The most common adverse events which contributed to more than 5% of the total events observed during the study were coughing (4.6% in olive group) and vertigo (5.9% in olive group). Less frequently, muscle discomfort and headache were reported (< 5% of the total events).

Serious adverse events and deaths

None reported.

Assessors comment

The safety profile of olive leaf extracts can be described as acceptable from the existing clinical studies (Susalit *et al.* 2011; Perrinjaquet-Moccetti *et al.* 2008) and from the use of products thereof on the

market. The safety results obtained from the clinical studies conducted so far show that the oral use of olive leaf extracts are well tolerated by most patients. The majority of adverse events were tolerably mild while the most common ones (5% of the total events observed during the studies) were coughing and vertigo (4.6% and 5.9% respectively in olive group). Less frequently, muscle discomfort and headache, were reported.

There are no reported drug-related serious or moderate adverse events. It is proposed in the literature that olive leaf extract preparations administrated to patients with biliary tract stones, could cause a risk of biliary colics through promoting the secretion of bile. Moreover a case of pollinosis, in the form of rhinitis or bronchial asthma has been reported (PDR for Herbal Medicines 2007).

5.4. Laboratory findings

None reported.

5.5. Safety in special populations and situations

The product is not suitable for patients with known hypersensitivity against the herbal substance, the plant family, the herbal preparation or the excipients of the final product.

Intrinsic (including elderly and children) / extrinsic factors

Olive leaf is not intended for use in children, while no restrictions are known for its use in elderly.

Drug interactions

No drug interactions have been reported.

Use in pregnancy and lactation

Olive leaf should not be used during pregnancy and lactation as no data are available on the use in pregnancy and lactation.

Overdose

No data available.

Drug abuse

No data available.

Withdrawal and rebound

No data available.

Effects on ability to drive or operate machinery or impairment of mental ability

No data available.

5.6. Overall conclusions on clinical safety

In the absence of data in special patient populations, *Olea* leaf is intended only for adults.
In the absence of data and in accordance with general medical practice, it is recommended not to use

the herbal medicinal products containing olive leaf during pregnancy and lactation. Fertility data are lacking.

The safety profile of olive leaf and olive extracts can be judged as good from the existing clinical data and from their long term use, more than 30 years, in the European market. The available literature, on pharmacological and toxicological studies, does not give reason for safety concerns.

As there is no available data on genotoxicity, carcinogenicity and reproductive toxicity on Oleae folium, the establishment of a Community List Entry is not possible for safety reasons.

6. Overall conclusions

The positive effects of olive leaf to enhance the excretion of urine and to support somehow the cardiovascular function (through its hypotensive activity) have long been recognised empirically. The use is made plausible especially by in *vitro* and *in vivo* pharmacological data. There are not many available clinical studies, using herbal preparations, containing the herbal substance of olive leaf. A very recent double-blind, randomised, parallel and active-controlled clinical study was conducted to evaluate the anti-hypertensive effect as well as the tolerability of olive leaf extract EFLA®943 in comparison with captopril in 148 patients, with stage-1 hypertension for 8 weeks (Susalit *et al.* 2011). The evaluation of all safety parameters and occurrence of adverse events showed that olive leaf extract was safe and tolerable in patients with stage-1 hypertension. In another study the same extract (EFLA®943), having antihypertensive actions in rats (Khayyal *et al.* 2002), was tested as a supplement in an open study including 40 borderline hypertensive monozygotic twins for 8 weeks. Concluding, the study confirmed the antihypertensive and cholesterol-lowering action in the tested humans (Perrinjaquet-Moccetti *et al.* 2008).

After discussions in both MLWP and HMPC it has been acknowledged that the tradition and the pharmacologically plausible threefold mild activity (diuretic, hypotensive, mild anti-hypercholesterolaemic) would be considered as beneficial for the cardiovascular system (function).

Safety concerns remain with respect to a cardiovascular indication, i.e. the demarcation between mild functional complaints and organic symptoms. More serious conditions may not be easily distinguished by patients. Even after exclusion of such conditions, it should be avoided that patients may be encouraged for self treatment, where clearly medical supervision and medically supervised medication is required. The HMPC endorsed therefore only a diuretic indication.

In conclusion, olive leaf's preparations can be accepted as traditional herbal medicinal products in the following indication:

Traditional herbal medicinal product used to promote the renal elimination of water, in mild cases of water retention.

The following herbal substances/preparations have been proposed

- fresh or dried leaves
- comminuted or powdered dried leaves for herbal tea
- powdered dried leaves.

After the acceptance of the above mentioned indication, two herbal preparations (Liquid extract (1:0.71-0.86 solvent: ethanol 96% V/V). and Dry extract (7.9-12:1), extraction solvent: ethanol 96% V/V.)), which are on the German market for more than 35 years were excluded from the monograph. The indication in Germany ("…to support the cardiovascular system") does not comply with the indication accepted by the HMPC.

The proposed herbal substance and herbal preparations have been traditionally used for more than 30 years. Therefore, on the basis of the well-known, long-lasting and traditional use of preparations of olive leaves in the folk medicine and as registered medicaments, the safe use can be stated.

In the absence of data in special patient populations, olive leaf is intended only for adults and elderly.

In the absence of data and in accordance with general medical practice, it is recommended not to use the herbal medicinal products containing olive leaf during pregnancy and lactation.

Thirty patients have been treated with water extracts of olive leaf (mainly 1,600 mg daily) from 15 days up to 12 weeks with a very good tolerability.

Another 188 patients have used ethanolic extracts of olive leaf for 8 weeks with no serious adverse effect (Susalit *et al.* 2011; Perrinjaquet-Moccetti *et al.* 2008). The majority of adverse effects reported in the existing clinical studies were tolerably mild (99.8%). The most common adverse events which contributed to more than 5% of the total events observed during the studies were coughing (4.6% in olive group) and vertigo (5.9% in olive group); less frequently, muscle discomfort and headache were reported. It is proposed in the literature that olive leaf extract preparations administrated to patients with biliary tract stones, could cause a risk of biliary colic through promoting the secretion of bile. Moreover one case of pollinosis, in the form of rhinitis or bronchial asthma has been also reported (PDR for Herbal Medicines 2007).

As there is no available data on genotoxicity, carcinogenicity and reproductive toxicity, the establishment of a Community List Entry is not possible for safety reasons.

Annex

List of references